

Thomas M. SchmidtDepartment of Microbiology
and Molecular Genetics,
Michigan State University,
East Lansing, Michigan, USA

The maturing of microbial ecology

Summary. A.J. Kluyver and C.B. van Niel introduced many scientists to the exceptional metabolic capacity of microbes and their remarkable ability to adapt to changing environments in *The Microbe's Contribution to Biology*. Beyond providing an overview of the physiology and adaptability of microbes, the book outlined many of the basic principles for the emerging discipline of microbial ecology. While the study of pure cultures was highlighted, provided a unifying framework for understanding the vast metabolic potential of microbes and their roles in the global cycling of elements, extrapolation from pure cultures to natural environments has often been overshadowed by microbiologists' inability to culture many of the microbes seen in natural environments. A combination of genomic approaches is now providing a culture-independent view of the microbial world, revealing a more diverse and dynamic community of microbes than originally anticipated. As methods for determining the diversity of microbial communities become increasingly accessible, a major challenge to microbial ecologists is to link the structure of natural microbial communities with their functions. This article presents several examples from studies of aquatic and terrestrial microbial communities in which culture and culture-independent methods are providing an enhanced appreciation for the microbe's contribution to the evolution and maintenance of life on Earth, and offers some thoughts about the graduate-level educational programs needed to enhance the maturing field of microbial ecology. [*Int Microbiol* 2006; 9(3):217-223]

Key words: microbial ecology · microbial diversity · genomics · graduate education

Address for correspondence:
Dept. of Microbiology and Molecular Genetics
6180 Biomedical and Physical Sciences Building
Michigan State University
East Lansing, MI 48824-4320, USA
Tel. +1-517-3556463, ext 1606.
Fax +1-517-3538957
Email: tschmidt@msu.edu

Introduction

Although plants and animals dominate our visual landscape of nature, microbes constitute a similar amount of biomass [26] and play pivotal roles in maintaining the viability of Earth's biosphere. In their celebrated book *The Microbe's Contribution to Biology* [12], A.J. Kluyver and C.B. van Niel introduced the scientific community to microbes and their critical roles in sustaining life with the following simple argument (*italics added*):

“Reliable estimates have shown that the amount of carbon dioxide consumed annually in the photosynthetic activity of

the green plants is such that the quantity of this gas present in the atmosphere would be exhausted within some 30 years, if it were not replenished. Even taking into account the important carbon dioxide reservoir present in the oceans as a buffer system, such an exhaustion should have occurred within historic times. It is, therefore, clear that the green plants can continue to grow only because the assimilated carbon is in some way reconverted into carbon dioxide. At first sight this will not present any difficulties to our “macrobiologist”; he will refer to the slow combustion of the vegetable remains by man and animals. However, several independent estimates tend to show that the annual carbon dioxide production by this means amounts to only about 5 percent of the annual carbon dioxide consumption by the

green plants. The conclusion seems inevitable that *the remaining 95 percent is produced by the mineralizing action of the microbe.*"

Although estimates of the magnitude of carbon flux and details of the processes that contribute to the cycling of carbon have been refined considerably in the past 50 years, microbes remain central catalytic agents in the global carbon cycle. Microbes also drive local and global cycles of nitrogen, sulfur, oxygen, phosphorus, and many of the transition metals: without the metabolic activity provided by microbes, plant or animal life could not be sustained. So what have we learned about the ecology of microbes since the publication of this book and where are the gaps in our scientific understanding?

The metabolic diversity of microbes

Kluyver and van Niel emphasized the core metabolic unity of life and described the impressive suite of auxiliary metabo-

lisms that permit the harvest of energy while coupling the oxidation and reduction of a wide variety of chemical reactions. Some of the common redox reactions catalyzed by microbes are presented in Fig. 1, in which the electron-donating half reactions (oxidations) are presented in the left hand tower, and electron-accepting half reactions (reductions) are presented in the tower on the right. Reactions are arranged from top to bottom in the order of most electronegative to electropositive, providing a simple way to determine whether coupled reactions will yield energy under standard conditions: draw a line between the half reactions on the towers and if the slope of the line is negative, the reaction is exothermic and there are almost certainly microbes capable of capturing the energy released in the reaction. Although environmental conditions such as pH, eH, and the concentration of reactants and products, alter the free energy available in these redox reactions, as a first approximation energy-yielding reactions can be easily identified by a downward sloping line.

In the past 50 years, there have been some spectacular discoveries of the types of energy-yielding reactions catalyzed by microbes, including the capacity to use insoluble

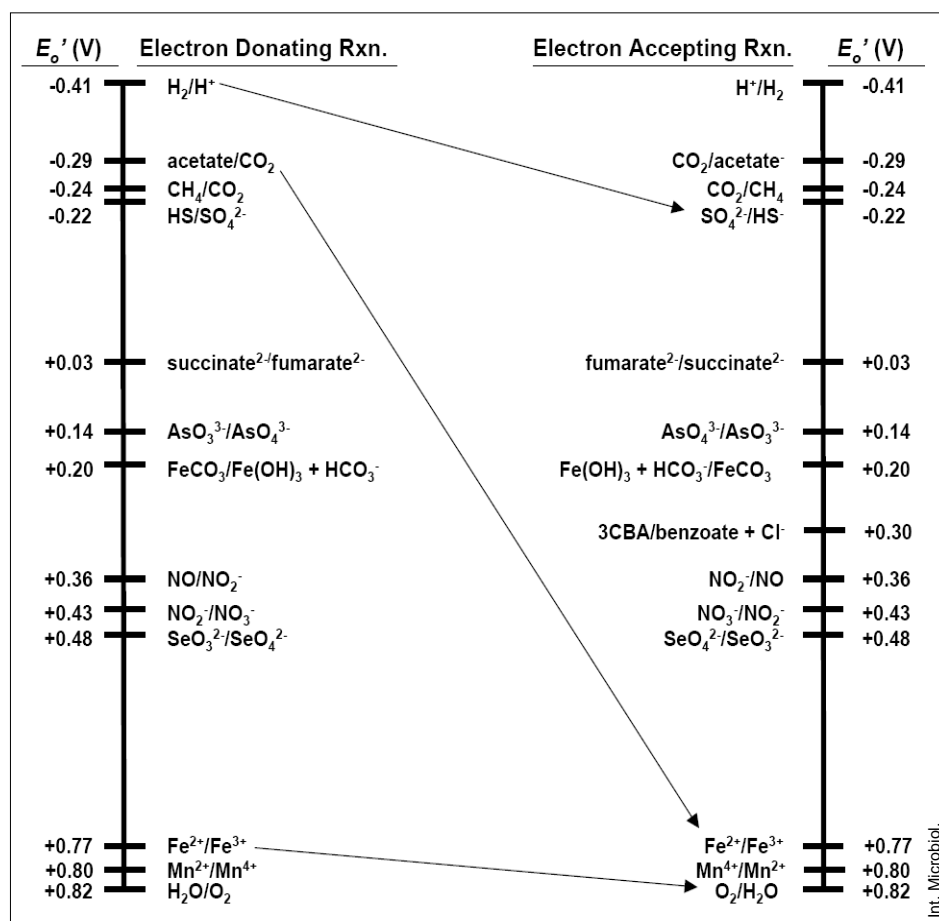
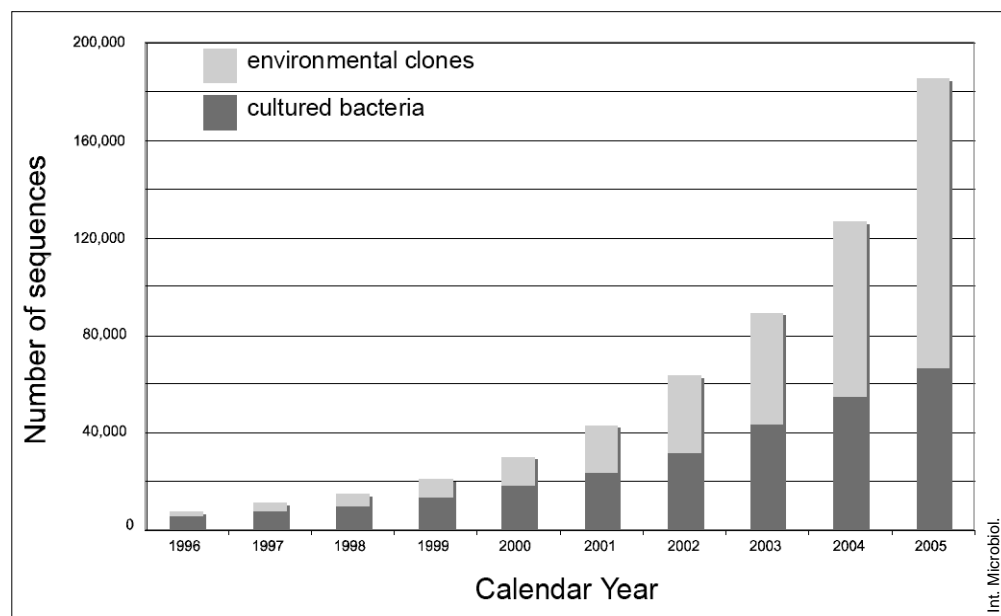


Fig. 1. Oxidation-reduction potentials of half reactions that are common electron donors and electron acceptors in microbial respiration. Downward sloping lines represent the coupling of half reactions that yield energy under standard conditions.

Fig. 2. Cumulative numbers of aligned 16S rRNA gene sequences available for analysis from the Ribosomal Database Project (<http://rdp.cme.msu.edu>) based on calendar year. The numbers of sequences from cultivated bacteria are presented in dark gray shading and the numbers of sequences derived from environmental samples are presented in light gray shading.



metal oxides as terminal electron acceptors (Fig. 1) [13] and the use of anthropogenic hydrocarbons as electron acceptors [8]. The microbially catalyzed, anaerobic oxidations of ammonia [23] and methane [24] have also expanded the types of microbial metabolisms in anoxic environments that contribute to global elemental cycles. It is in terms of their metabolism that microbes have revealed incredible diversity as compared to the uniform types of metabolism found throughout the plant and animal kingdoms. Nevertheless, understanding the evolutionary diversity underlying this metabolic diversity required a systematic means to determine the evolutionary relatedness of microbes.

The evolutionary diversity of microbes

In a seminal article demonstrating that sequences of macromolecules could be used to infer the evolutionary relationships among organisms, Emile Zuckerkandl and Linus Pauling provided a conceptual framework for molecular phylogenetic analysis [28]. However, it was not until Carl Woese applied the methods of molecular phylogeny to ribosomal RNA sequences that the remarkable evolutionary diversity of the microbial world was realized [27]. The early determination of rRNA sequences required that the organism under investigation be grown in pure culture, where the incorporation of radioactive phosphorus into ribosomal RNA labeled the RNA for oligonucleotide cataloging. But microbiologists long recognized that the proportion of microbes that form colonies on solid media represents a small fraction of the

microbes visible microscopically in most environments [25]. The discrepancy between the number of visible microbe and those that form colonies on solid media was dubbed “The Great Plate Count Anomaly” [21], and represented one of the most severe constraints on efforts to determine the composition of natural microbial communities.

Fortunately, the introduction of DNA-based approaches for determining the composition of microbial communities provided a means for assessing the composition of microbial communities without the limitations imposed by cultivation [15]. In the two decades since the introduction of cultivation-independent surveys of the environment, there have been dozens of new phyla discovered that lack cultured representatives [14].

Molecular surveys of microbial communities have become a routine dimension of studies of microbial communities, resulting in an explosion of 16S rRNA gene sequences derived directly from environmental samples (Fig. 2). Analyses of molecular surveys now provide the opportunity to compare the composition of microbial communities and lead to hypotheses about how changes in composition result in changes in their function.

Linking the structure and function of microbial communities

Over the past decade, molecular-based approaches have revealed a enormous phylogenetic diversity within the microbial world that is not yet represented in culture [1,9]. This

impression has come almost entirely by retrieval of small subunit (SSU) rRNA sequence information, which provides a phylogenetic context in which to quantify such a diversity, and typically involves cloning and sequencing SSU rRNA-encoding genes. While phylogenetically informative, such clones do not always allow predictions to be made about the physiological properties of a source organism, unless the source was a member of a phylogenetic group exhibiting a distinctive type of metabolism, e.g., a cyanobacterium, a methanogenic archaeon. This is especially true for clones from phylogenetic groups that are seriously underrepresented in culture collections. How do we move beyond phylogenetic trees that reveal the evolutionary diversity of microbes present in an environment and advance our understanding of their metabolic potential?

In an effort to increase the information retrieved from environmentally cloned SSU rDNA, a number of investigators have exploited cloning vectors that can accommodate large fragments (40 kb) of genomic DNA that include numerous genes adjacent to the phylogenetic signature, i.e., the SSU rDNA. Stein and colleagues [22] used a fosmid vector to obtain 35- to 45-kb fragments of genomic DNA from a marine picoplankton community containing an abundant, but yet-uncultured, marine crenarchaeote. Sequencing of regions adjacent to the SSU rDNA locus revealed a number of genes, including an RNA helicase and one involved in heme biosynthesis, thereby providing a glimpse into the physiological potential of the source organism. Béjà and co-workers used a bacterial artificial chromosome (BAC) vector to clone genomic DNA fragments up to 150 kb in size from marine planktonic assemblages [3]. Among the genes flanking the SSU rDNA of one such clone, derived from a γ -proteobacterium, was a gene encoding a bacterial version of rhodopsin, implying a novel type of phototrophy occurring in the sea and suggesting obvious strategies that might be used to obtain such organisms in culture [2]. BAC vectors were also used to clone large fragments of DNA from soil microbes [18] and revealed an assortment of genes encoding degradative and biosynthetic enzymes, as well as antibiotic production. A holistic view of the way these nucleic-acid-based approaches help link the structure and function of microbial communities is presented in Fig. 3. Stable-isotope probing [16] provides another approach to link structure and function. Analysis of genome composition, for instance through analysis of the redundancy of ribosomal RNA genes, has also been used to identify ecological strategies of bacteria [11].

Many of the applications of genomic sciences to microbial ecology have been pioneered in marine systems and are the focus of an accompanying article in this issue; only recently have they been applied to terrestrial ecosystems.

This has been due in part to the difficulty of purifying nucleic acids from the soil matrix, but the effort will likely be worth the investment of time and energy because the biodiversity harbored in soils of the Earth is staggering [4]. One gram of soil contains up to 1×10^{10} organisms, representing as many as one million bacterial species [7]. The combined acreage of soil contains 26×10^{28} Bacteria and Archaea that harbor 26 Pg of C, 6.2 Pg of N, and 0.65 Pg of P [26]—huge amounts, even when compared to the total amount of these elements in terrestrial plants (559 Pg C, 10 Pg N, 1.05 Pg P). The importance of microbes as a component of the Earth's terrestrial biomass, and their power to influence global ecosystems, is derived from their sheer numbers and the diverse array of biochemical reactions they catalyze. In addition to their impact on terrestrial habitats, soil microbes also affect atmospheric chemistry and global climate by influencing budgets of atmospheric gases, including CO_2 , CH_4 , H_2 , N_2O , and NO [5,10]. Recent results from long-term studies [17] suggest that varying management practices have major impacts on fluxes of greenhouse gases, i.e., CO_2 , N_2O , and CH_4 , and carbon sequestration. While it is generally accepted that microbial communities in soil are critical to the productivity and health of the biosphere [6], and the documented diversity in these communities is spectacular, the challenge of understanding how the function of a community is related to its structure remains.

Given the magnitude of microbial diversity in both terrestrial [7] and aquatic ecosystems [19] and the integral role of microbes in virtually all environments, successful investigation of the structure and function of microbial communities requires enhanced opportunities in graduate education.

Rethinking graduate education in microbial ecology

It is a disturbing reality that we have only fragmentary understanding of the enormous microbial diversity that exists on our planet: This applies not merely to microbes living in extreme environments and which would be expected to possess unusual and perhaps not yet fully characterized properties, but also to those in mundane habitats—a gram of soil, a milliliter of seawater. Yet the metabolic activity of these microbes is essential to life on Earth: without the unique roles filled by microbes in the global recycling of carbon, sulfur, and nitrogen, plants and animals could not survive. Despite their importance, many such microbes have continued to elude conventional isolation and cultivation techniques. Fortunately, the same developments in molecular biology presented above that have enabled us to recognize

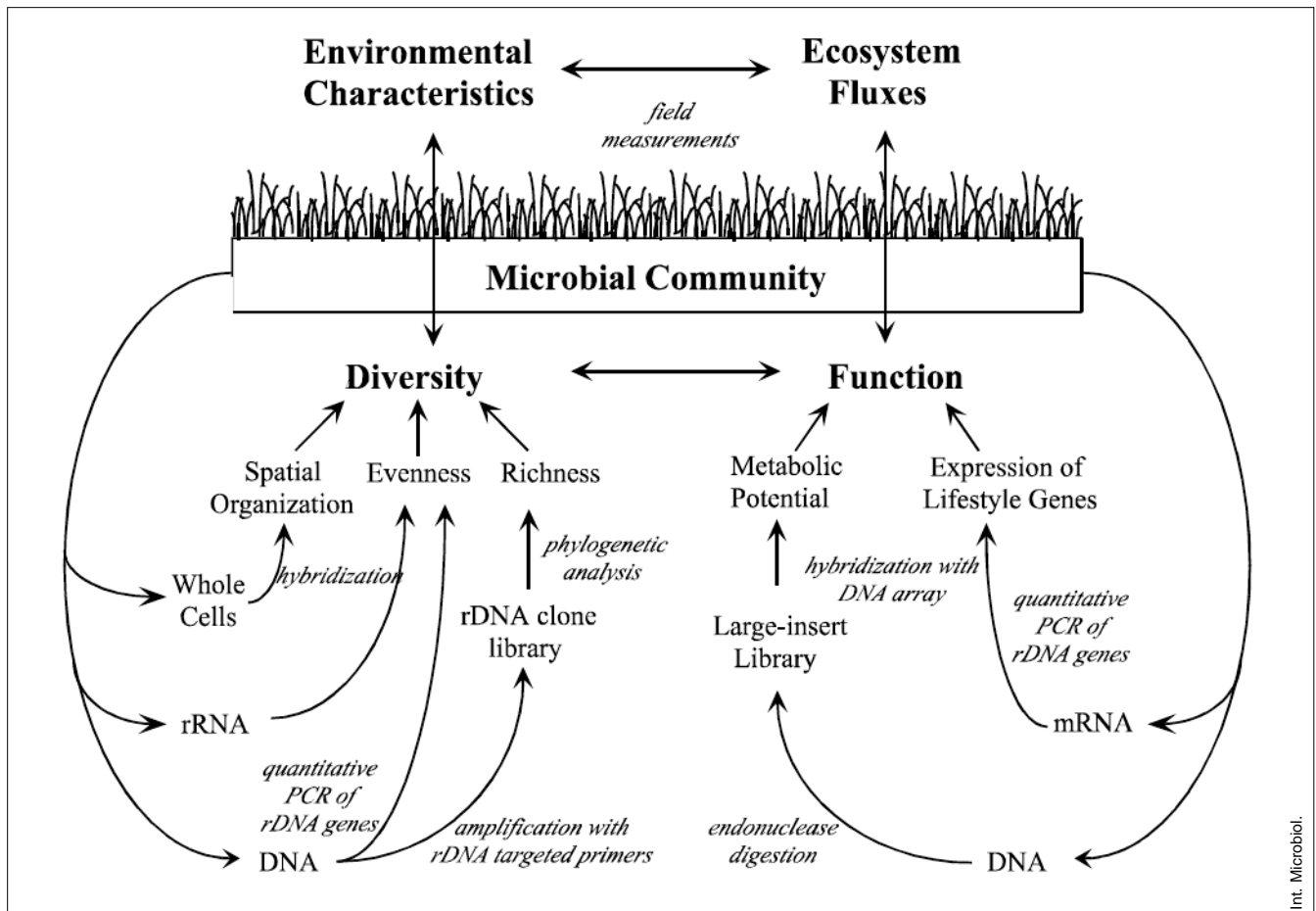


Fig. 3. Integration of nucleic-acid-based approaches to linking the structure and function of microbial communities.

such untapped microbial diversity are now supplying the very tools necessary to quantify, monitor, dissect, and understand it. However, the effective application of such technology has created a need for a 'new breed' of microbiologist: a hybrid individual who is as much at home annotating a genome sequence as evaluating stable isotope data; as conversant in microbial bioenergetics as in gene cloning strategies. Such individuals need a global perspective and experience in functioning within multidisciplinary, often international, teams, because the most important questions that confront us today are global in scope and often too complex to be addressed by individuals or small research teams.

Several factors will likely drive much of the research in microbial ecology over the next decade, including:

(i) Recognition of the enormous biomass and diversity of microbial life on our planet.

(ii) The unsettling acknowledgement that most of this diversity is poorly understood, and yet almost certainly affects global-level changes and environmental stability on our planet.

(iii) The steadily growing facility with which microbial genomes and communities of microbes can be sequenced.

If this vision is accurate, then traditional graduate programs in microbiology must be re-tooled to educate the next generation of microbiologists, who must be broadly trained, versatile, and capable of working in multidisciplinary teams, and who can think holistically—from the genome level to global-level processes. Graduate students must also have the opportunity to gain research experience in the laboratory *and* in the field—ideally, in habitats representative of those that dominate the Earth, i.e., terrestrial, freshwater, and marine. In this way, they can gain an appreciation for the various scales at which microbial processes are occurring, as well as an understanding of the most important questions and the methodologies used to address them. It would be ideal for graduate students to be exposed to nonlinear biological systems and mathematical methods for teasing out potentially deterministic relationships from "noise" as they begin to link the genomic and phylogenetic diversity of microbial life on Earth with its function and planetary impact.

Contemporary graduate training programs designed to accomplish this do not currently exist, and it is not difficult to understand why. It is rare that a single department, or university, has the blend of expertise and resources to provide such a robust training experience on its own. Nevertheless, atop a list of recommendations made in a recent report by the American Academy of Microbiology [20] was a need to develop training opportunities to advance the emerging new field of "ecogenomics" (the intersection of ecology and genome science) as a means to explore the many levels of biological organization that sustain the biosphere. Such training programs will require an integration of knowledge from a number of disciplines, including microbiology, ecology, evolutionary biology, genomic science, biogeochemistry, mathematics and bioinformatics.

As human civilization leads to ever increasing perturbations of the environment, we need more than ever to understand the essential role of microbes in maintaining a habitable Earth. The recognition of such a need will ideally lead to new educational opportunities for scientists such that they will be in a position to advance our understanding of the microbial world and its effect on the Earth's biosphere, hydrosphere and atmosphere.

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References

- Amann RI, Ludwig W, Schleifer K-H (1995) Phylogenetic identification and in situ detection of individual microbial cells without cultivation. *Microbiol Rev* 59:143-169
- Béjà O, Aravind L, Koonin EV, Suzuki MT, Hadd A, Nguyen LP, Jovanovich S, Gates CM, Feldman RA, Spudich JL, Spudich EN, DeLong EF (2000) Bacterial rhodopsin: Evidence for a new type of phototrophy in the sea. *Science* 289:1902-1906
- Béjà O, Suzuki MT, Koonin EV, Aravind L, Hadd A, Nguyen LP, Villacorta R, Amjadi M, Garrigues C, Jovanovich SB, Feldman RA, DeLong EF (2000) Construction and analysis of bacterial artificial chromosome libraries from a marine microbial assemblage. *Env Microbiol* 2:516-529
- Buckley DH, Schmidt TM (2002) Exploring the biodiversity of soil. A microbial rainforest. In: Staley JT, Reysenbach A.-L. (eds) *Biodiversity of microbial life*. Wiley-Liss, New York
- Conrad R (1996) Soil microorganisms as controllers of atmospheric trace gasses (H₂, CO, CH₄, OCS, N₂O, and NO). *Microbiol Rev* 60:609-640
- Copley J (2000) Ecology goes underground. *Nature* 406:452-454
- Gans J, Wolinsky M, Dunbar J (2005) Computational improvements reveal great bacterial diversity and high metal toxicity in soil. *Science* 309:1387-1390
- He J, Sung Y, Krajmalnik-Brown R, Ritalahti KM, Löffler FE (2005) Isolation and characterization of *Dehalococcoides* sp. strain FL2, a trichloroethene (TCE)- and 1,2-dichloroethene-respiring anaerobe. *Environ Microbiol* 7:1442-1450
- Hugenholtz P, Goebel BM, Pace NR (1998) Impact of culture-independent studies on the emerging phylogenetic view of bacterial diversity. *J Bacteriol* 180:4765-4774
- IPCC (2001) *Climate change 2001: The scientific basis*. Cambridge University Press, Cambridge, UK
- Klappenbach J, Dunbar JM, Schmidt TM (2000) rRNA operon copy number reflects ecological strategies in bacteria. *Appl Environ Microbiol* 66:1328-1333
- Kluyver AJ, van Niel CB (1956) *The Microbe's Contribution to Biology*. Harvard University Press, Cambridge, MA
- Lovley DR, Holmes DE, Nevin KP (2004) Dissimilatory Fe(III) and Mn(IV) reduction. *Adv Microb Physiol* 49:219-286
- Pace NR (1997) A molecular view of microbial diversity and the biosphere. *Science* 276:734-740
- Pace NR, Stahl DA, Lane DJ, Olsen GJ (1986) The analysis of microbial populations by ribosomal RNA sequences. *Adv Microb Ecol* 9:1-55
- Radajewski S, Ineson P, Parekh NR, Murrell JC (2000) Stable-isotope probing as a tool in microbial ecology. *Nature* 403:646-649
- Robertson GP, Paul EA, Harwood RR (2000) Greenhouse gases in intensive agriculture: contributions of individual gases to the radiative forcing of the atmosphere. *Science* 289:1922-1925
- Rondon MR, August PR, Betterman AD, Brady SF, Grossman TH, et al. (2000) Cloning the soil metagenome: a strategy for accessing the genetic and functional diversity of uncultured microorganisms. *Appl Environ Microbiol* 66:2541-2547
- Sogin ML, Morrison HG, Huber JA, Welch DM, Huse SM, Neal PR, Arrieta JM, Herndl GJ (2006) Microbial diversity in the deep sea and the underexplored "rare biosphere". *Proc Natl Acad Sci USA* 103:12115-12120
- Stahl DA, Tiedje JM (2002) Microbial ecology and genomics: a crossroads of opportunity. *American Academy of Microbiology*, Washington, DC, p 28
- Staley JT, Konopka A (1985) Measurement of in situ activities of non-photosynthetic microorganisms in aquatic and terrestrial habitats. *Annu Rev Microbiol* 39:321-346
- Stein JL, Marsh TL, Wu KY, Shizuya H, DeLong EF (1996) Characterization of uncultivated prokaryotes: isolation and analysis of a 40-kilobase-pair genome fragment from a planktonic marine archaeon. *J Bacteriol* 178:591-599
- Strous M, Fuerst JA, Kramer EH, Logemann S, Muyzer G, van de Pas-Schoonen KT, Webb R, Kuenen JG, Jetten MS (1999) Missing lithotroph identified as new planctomycete. *Nature* 400:446-449
- Valentine DL (2002) Biogeochemistry and microbial ecology of methane oxidation in anoxic environments: a review. *Ant Leeuw* 81:271-282
- Ward DM, Bateson MM, Weller R, Ruff-Roberts AL (1992) Ribosomal RNA analysis of microorganisms as they occur in nature. *Adv Microb Ecol* 12:219-286
- Whitman WB, Coleman DC, Wiebe WJ (1998) Prokaryotes: The unseen majority. *Proc Natl Acad Sci USA* 95:6578-6583
- Woese CR, Kandler O, Wheelis ML (1990) Towards a natural system of organisms: Proposal for the domains Archaea, Bacteria, and Eucarya. *Proc Natl Acad Sci USA* 87:4576-4579
- Zuckerkind E, Pauling L (1965) Molecules as documents of evolutionary history *J Theor Biol* 8:357-366

La madurez de la ecología microbiana

Resumen. Con su libro *The Microbe's Contribution to Biology*, A.J. Kluyver y C.B. van Niel mostraron a muchos científicos la capacidad metabólica excepcional de los microbios y su notable habilidad para adaptarse a los cambios ambientales. Además de una descripción de la fisiología y de la adaptabilidad de los microbios, el libro destacaba muchos de los principios básicos de la ecología microbiana, que era entonces una disciplina emergente. Mientras el estudio de los cultivos puros ocupaba un lugar destacado, proporcionando un marco unitario para la comprensión del amplio potencial metabólico de los microbios, y de su función en el ciclo global de los elementos, la extrapolación de cultivos puros a ambientes naturales fue eclipsada a menudo por la incapacidad de los microbiólogos de cultivar muchos de los microbios observados en ambientes naturales. La combinación de enfoques genómicos está proporcionando una visión del mundo microbiano independiente del cultivo, lo que revela una comunidad de microbios más diversa y más dinámica de lo que se había considerado originalmente. A medida que los métodos para determinar la diversidad de las comunidades microbianas se hacen más accesibles, un reto importante para los ecólogos microbianos es relacionar la estructura de las comunidades microbianas naturales con sus funciones. Este artículo presenta varios ejemplos que provienen de estudios de comunidades microbianas acuáticas y terrestres, en los cuales tanto los métodos dependientes de cultivo como los que no lo son, amplían la apreciación de la contribución de los microbios a la evolución y al mantenimiento de la vida en la Tierra. También ofrece algunas reflexiones sobre la necesidad de que las licenciaturas realcen el campo ya maduro de la ecología microbiana. [Int Microbiol 2006; 9(3):217-223]

Palabras clave: ecología microbiana · diversidad microbiana · genómica · formación universitaria

A maturidade da ecologia microbiana

Resumo. Com seu livro *The Microbe's Contribution to Biology*, A.J. Kluyver e C.B. van Niel mostraram a muitos cientistas a capacidade metabólica excepcional dos micróbios e sua notável habilidade para adaptar-se às mudanças ambientais. Além de uma descrição da fisiologia e da adaptabilidade dos micróbios, o livro destacava muitos dos princípios básicos da ecologia microbiana, uma disciplina em surgimento. Enquanto o estudo das culturas puras ocupava uma posição de destaque, proporcionando um marco único para a compreensão do amplo potencial metabólico dos micróbios e de sua função no ciclo global dos elementos, a extrapolação de culturas puras para ambientes naturais foi eclipsada a miúdo pela incapacidade dos microbiologistas de cultivar muitos dos micróbios de ambientes naturais. A combinação de enfoques genômicos está proporcionando uma visão cultural independente do mundo microbiano, revelando uma comunidade mais diversa e dinâmica de micróbios da qual se tinha considerado originalmente. Como os métodos para determinar a diversidade de comunidades microbianas são cada vez mais acessíveis, um desafio importante para os ecólogos microbianos é relacionar a estrutura das comunidades microbianas naturais com suas funções. Este artigo apresenta vários exemplos que provêm de estudos das comunidades microbianas aquáticas e terrestres, nos quais os métodos de cultivo e de cultivo independente ampliam a valorização da contribuição dos micróbios à evolução e à manutenção da vida na Terra. Também oferece algumas reflexões sobre a necessidade de que os programas de estudo das licenciaturas contemplem o campo já maduro da ecologia microbiana. [Int Microbiol 2006; 9(3):217-223]

Palavras chave: ecologia microbiana · diversidade microbiana · genômica · formação universitária